Award #0313708

Coral Reef Genomics:
A Genome-wide Approach to the Study of Coral Symbiosis

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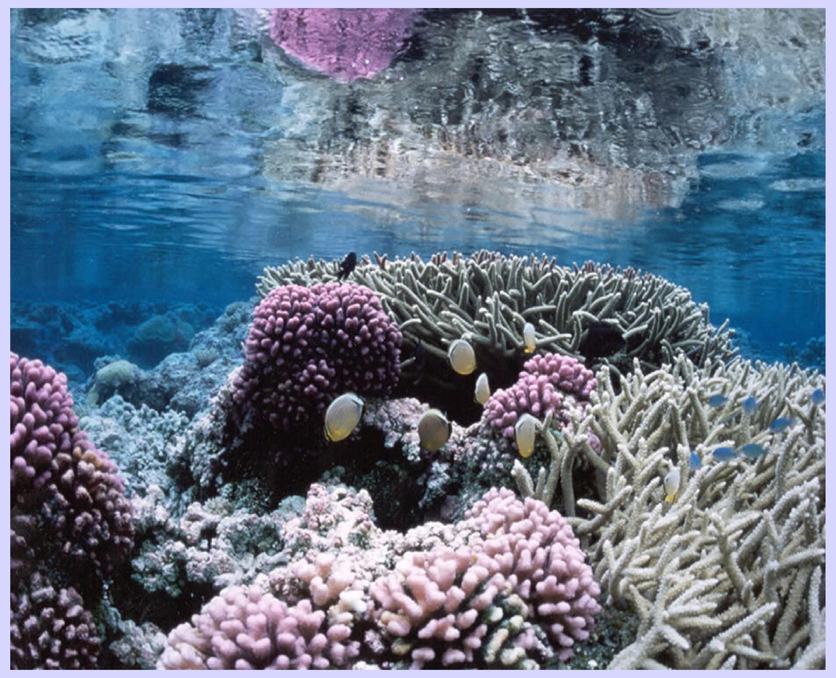
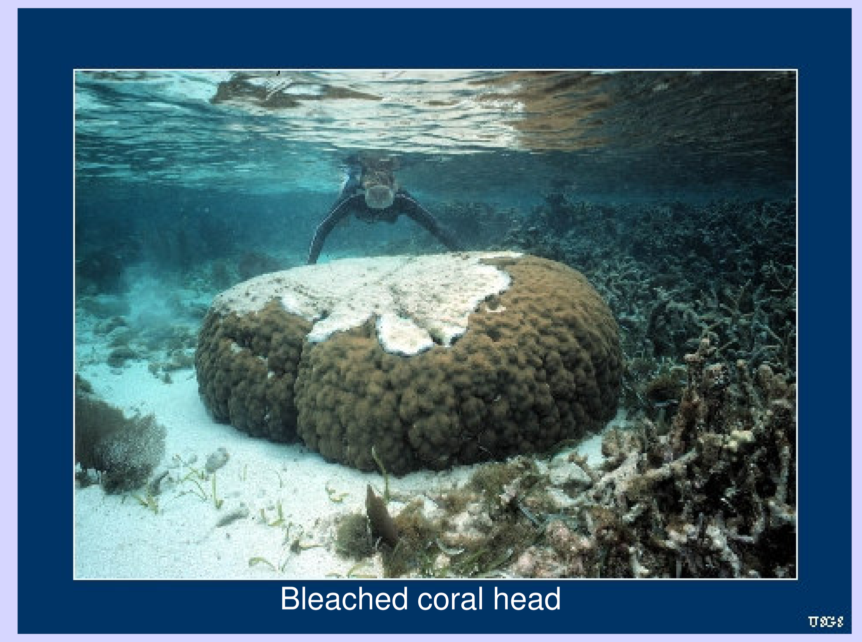


INTRODUCTION

Coral reefs are among the most beautiful and biodiverse ecosystems, upon which 500 million people depend for food, coastal protection, and other resources[1]. At the heart of the reef ecosystem is a symbiosis between corals and endosymbiotic dinoflagellates (*Symbiodinium* spp.). The presence of the photosynthetic *Symbiodinium* within coral tissues promotes tight nutrient recycling, enhanced production of coral skeleton, and increased biomass, which in turn supports the reef. Yet coral reefs are threatened by the declining health of the marine environment: coral bleaching and coral disease are causing the world's reefs to suffer drastic declines in coral cover. In the Caribbean alone, the primary reef-building coral, *Acropora palmata* has suffered >80% decline over the past 30 years[1]. We want to understand how this symbiosis is established, how it is maintained, and how it breaks down under conditions of environmental stress.

Brown spherical dinoflagellate symbionts within host tentacle



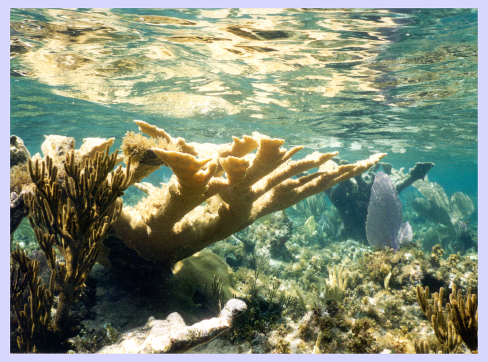
Bleached coral head

QUESTIONS

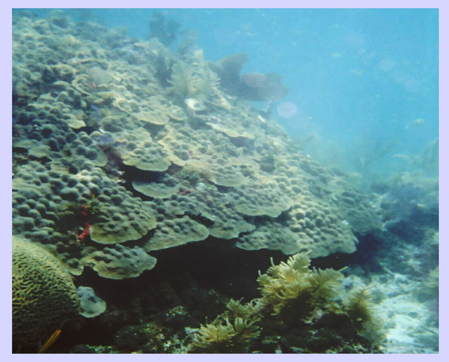
How do coral hosts and dinoflagellate symbionts establish and regulate the symbiosis?

- What genes or pathways are involved in the establishment of the symbiosis?
- Does the host initiate an immune response? Do the symbionts evade the response?
- How is gene expression affected by environmental conditions, symbiont strain, etc.

Study organisms



Acropora palmata



Montastraea faveolata

EXPERIMENTAL APPROACH

Experimentally initiate the symbiosis, sample RNA from each partner throughout the onset of symbiosis

Create libraries of expressed genes, design cDNA microarrays to identify symbiosis-related genes

Construct large-insert genomic BAC libraries to examine genomic context of symbiosis-related genes

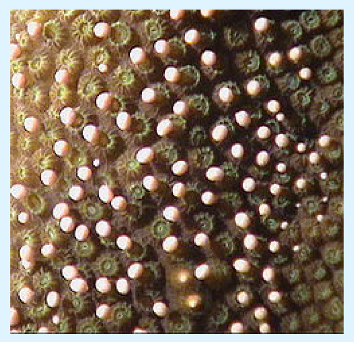
EDUCATION COMPONENT coordinated through CALIFORNIA ACADEMY OF SCIENCES

♦ Master's student developing a coral-related curriculum lending kit for schools

♦ Careers in Science program: development of a hands-on coral reef demonstration for public use at the museum

♦ Docent Program: docent education on coral symbiosis

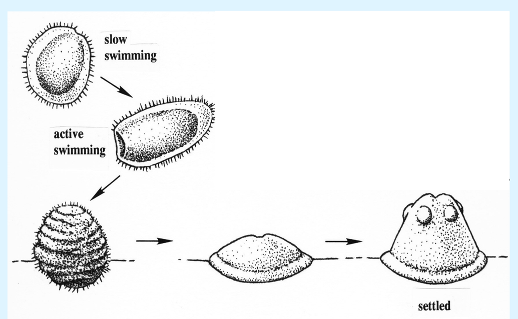
Field collections and experiments



Corals release pink egg/sperm bundles

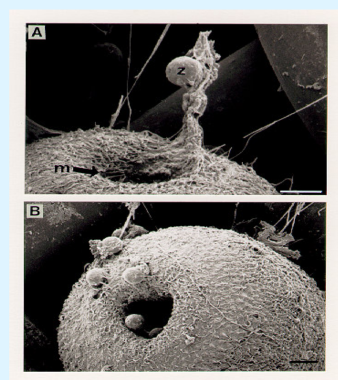
Mesh nets capture egg/sperm bundles

Eggs / sperm stocks established for fertilization experiments

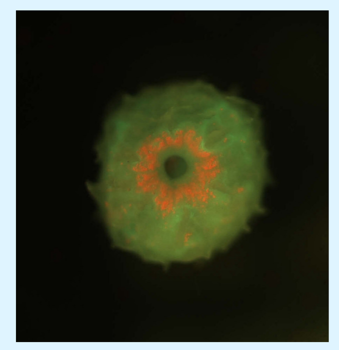


Fertilized eggs develop into larvae, and metamorphose into polyps

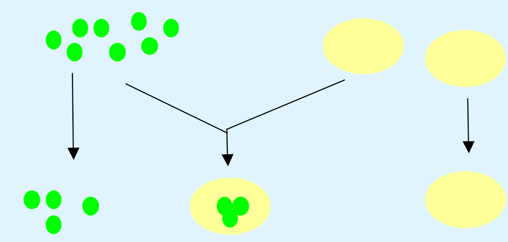
Cultures of *Symbiodinium* strains used for experimental infections



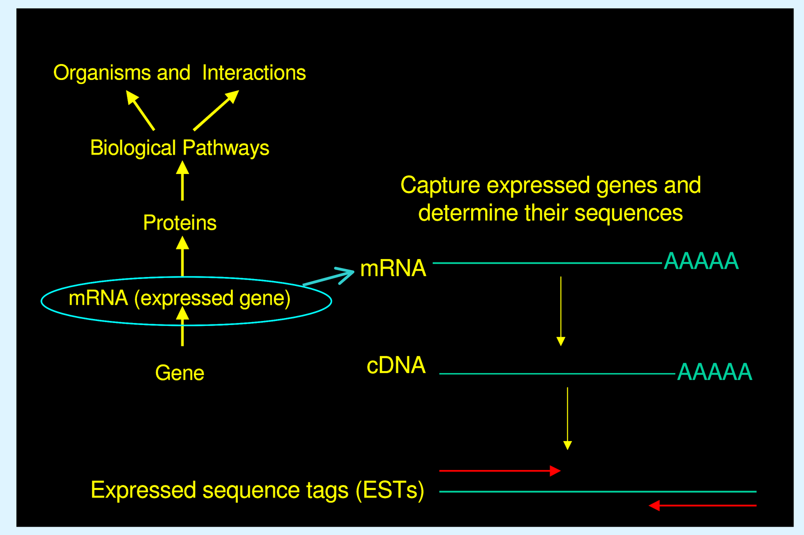
symbionts enter host gastric cavity (photo of *Fungia scutaria*)



Acropora palmata larva (green) infected with Symbiodinium (red)



Sampling methods: Experimentally infect larvae with *Symbiodinium* and collect samples of the symbiotic partners and non-symbiotic partners throughout the infection process



Extract RNA from all samples for construction of cDNA libraries

cDNA library construction and EST Sequencing

1. Sample genes from both hosts from as many stages of symbiosis as possible
2. Sequence a portion of each library to identify some of the genes expressed at each stage
3. 16 libraries will be constructed, representing both partners in the non-symbiotic and symbiotic conditions for two coral species and their dominant strains of *Symbiodinium*

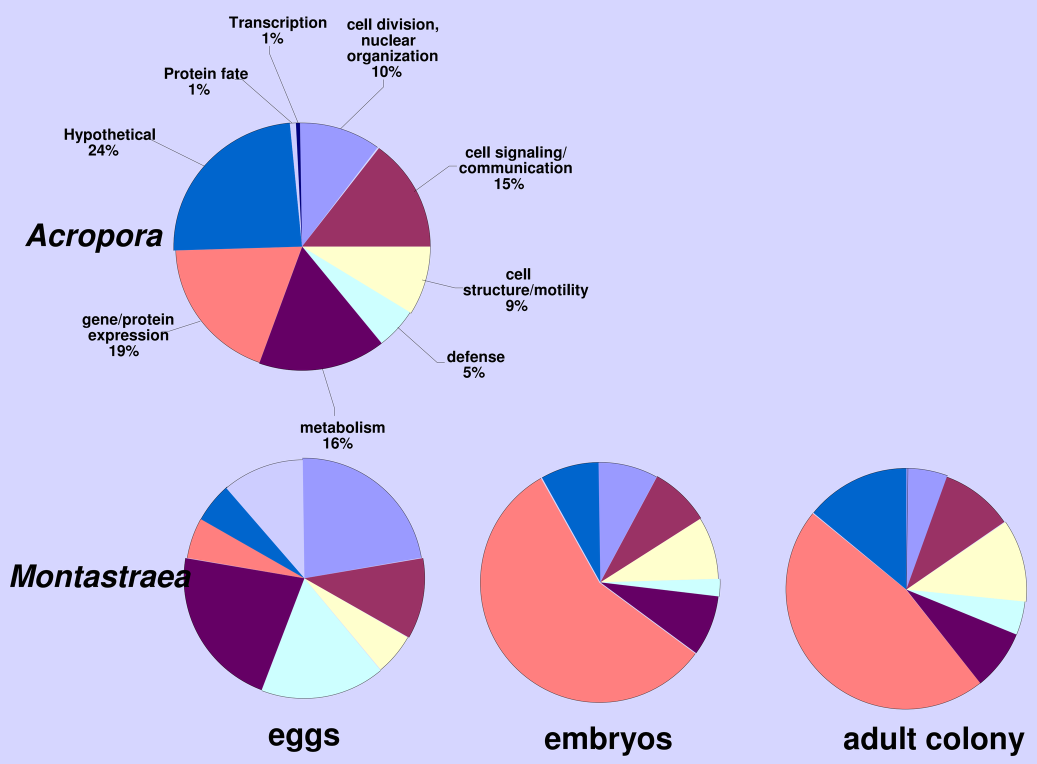
Symbiosis Status	Source of RNA	Number of ESTs *M. faveolata*	*A. palmata*
NS	Coral Eggs	1536	3840
NS	Coral Embryos	1536	1536
NS	Coral Larvae	1536	1536
S	Coral Larvae	1536	1536
S	Coral adult colony	2304	In progress
NS	*Symbiodinium* grown in culture	In progress	In progress
S	*Symbiodinium* isolated from larvae	In progress	In progress
S	Native *Symbiodinium* isolated from adult colony	In progress	In progress
		Total ESTs = 16896	
NS= Nonsymbiotic, S= symbiotic			

Status of library construction and EST sequencing for the planned target stages for both host species and symbiont strains

Annotation of ESTs

Obtain information about the potential functions of genes

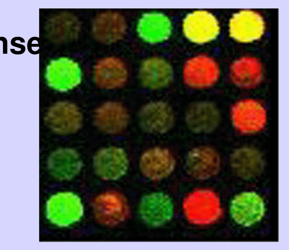
1. Gene identity as deduced from BLAST searches
2. Grouping of genes into larger-order biological processes



Comparison of larger-order biological processes between 4 cDNA libraries from two species and 3 developmental stages

Microarray design and experiments

Identify symbiosis-related genes by comparing gene expression patterns prior to and throughout the onset and maturation of the symbiosis



1. Select cDNAs to include in microarrays based on functional information obtained from EST annotation
2. Probe the microarrays with RNA samples at various timepoints of the symbiosis
3. Identify the relative expression of genes at different stages

	Fold Change	Best Blast hit Gene Identity	*Organism*	Accession #	E-value	Score
Up in adults	22.48	beta actin	*Aiptasia pulchella*	AAQ62633.1	$1x10^{-13}$	73.9
	15.94	catalase	*Drosophila melanogaster*	NP_536731.1	$1x10^{-14}$	81.3
	15.77	Tubulin alpha chain	*Gallus gallus*	P02552	$1x10^{-85}$	315
	12.92	ubiquitin/ribosomal protein S27a fusion protein	*Branchiostoma belcheri tsingtaunese*	AAL55470.1	$1x10^{-60}$	231
	9.03	histone H4	*Styela plicata*	JN0688	$1x10^{-42}$	172
	7.67	vitellogenin	*Pseudocentrotus depressus*	AAK57983	$1x10^{-12}$	74
	4.34	dynein	*Rattus norvegicus*	NP_062099.2	$1x10^{-85}$	317
	2.43	ankyrin 1	*Homo sapiens*	B35049	$1x10^{-42}$	175
	1.88	no hit				
Up in eggs	2.76	protein tyrosine phosphatase type IVA	*Homo sapiens*	NP_003454.1	$1x10^{-55}$	216
	2.66	no hit				
	2.54	chromatin-binding protein (*Drosophila* HP1 beta)	*Danio rerio*	NP_956040	$1x10^{-12}$	71.2
	2.45	estrogen receptor binding protein	*Homo sapiens*	AAQ95169	$1x10^{-33}$	142
	2.34	no hit				

Relative gene expression levels in *M. faveolata* eggs vs. adult corals (p<0.1)

References: [1]Status of Coral Reefs of the World: 2004 Vol. 1. C. Wilkinson, ed.

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